

Application No.: 10/033,571

Docket No.: 29853/37702

**AMENDMENTS TO THE CLAIMS**

**In the Claims:**

Please amend independent claim 70 to incorporate the limitation of claim 73 which is hereby cancelled. Claims 71, 72, 74-78, 82, 88-90 and 94-98 are also amended to delete the reference to "for therapeutic use." The following listing of claims will replace all prior versions, and listings, of claims in the application:

70. (Currently Amended) A method for making a purified adenovirus composition ~~for therapeutic use~~ comprising:

- a) growing host cells in a media;
- b) providing nutrients to said host cells by perfusion, fed batch or automated roller bottles;
- c) infecting said host cells with an adenovirus;
- d) lysing said host cells to provide a cell lysate comprising adenovirus; and
- e) purifying adenovirus from said lysate by a process other than the use of cesium chloride density gradient centrifugation to provide a pharmaceutically acceptable purified adenovirus composition having a contaminating nucleic acid concentration of less than 0.8 ng/ml.

71. (Currently Amended) The method of claim 70, wherein the purified adenovirus composition ~~for therapeutic use~~ comprises 70% +/- 10% of the starting PFU.

Application No.: 10/033,571

Docket No.: 29853/37702

72. (Currently Amended) The method of claim 70, wherein the purified adenovirus composition ~~for therapeutic use~~ comprises a substantially purified adenovirus composition.

73. (Cancelled)

74. (Currently Amended) The method of claim 70, wherein the purified adenovirus composition ~~for therapeutic use~~ has a contaminating nucleic acid concentration of less than 0.2 ng/ml.

75. (Currently Amended) The method of claim 70, wherein the purified adenovirus composition ~~for therapeutic use~~ has an A<sub>260</sub>/A<sub>280</sub> ratio of between about 1.2 and 1.3.

76. (Currently Amended) The method of claim 70, wherein the purified adenovirus composition ~~for therapeutic use~~ has an A<sub>260</sub>/A<sub>280</sub> ratio of 1.27 +/- 0.03.

77. (Currently Amended) The method of claim 70, wherein the purified adenovirus composition ~~for therapeutic use~~ has a contaminating nucleic acid concentration of less than 0.8 ng/ml and an A<sub>260</sub>/A<sub>280</sub> ratio of between about 1.2 and 1.3.

Application No.: 10/033,571

Docket No.: 29853/37702

78. (Currently Amended) The method of claim 70, wherein the purified adenovirus composition ~~for therapeutic use~~ has a BSA content below the detection level of a western blot assay.

79. (Previously Presented) The method of claim 70, wherein the media is serum-free.

80. (Previously Presented) The method of claim 70, wherein the host cells are grown in a bioreactor.

81. (Previously Presented) The method of claim 70, wherein the host cells are grown on microcarriers.

82. (Currently Amended) The method of claim 70, wherein said purified adenovirus composition ~~for therapeutic use~~ comprises an adenoviral vector encoding an exogenous gene construct.

83. (Previously Presented) . . . The method of claim 82, wherein said exogenous gene construct is operatively linked to a promoter.

84. (Previously Presented) The method of claim 83, wherein said promoter is SV40 IE, RSV LTR, β-actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

Application No.: 10/033,571

Docket No.: 29853/37702

85. (Previously Presented) The method of claim 82, wherein said exogenous gene construct encodes a therapeutic gene.

86. (Previously Presented) The method of claim 85, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*, antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-1, MEN II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, GM-CSF, G-CSF, thymidine kinase or p53.

87. (Previously Presented) The method of claim 86, wherein said therapeutic gene encodes p53.

88. (Currently Amended) The method of claim 70, wherein said purified adenovirus composition for therapeutic use is a replication-incompetent adenovirus.

89. (Currently Amended) The method of claim 88, wherein said purified adenovirus composition for therapeutic use is lacking at least a portion of the E1-region.

Application No.: 10/033,571

Docket No.: 29853/37702

90. (Currently Amended) The method of claim 89, wherein the purified adenovirus composition for therapeutic use is lacking at least a portion of the E1A and/or E1B region.

91. (Previously Presented) The method of claim 70, wherein said host cells are capable of complementing replication.

92. (Previously Presented) The method of claim 70, wherein said host cells are 293 cells.

93. (Previously Presented) The method of claim 70, wherein said lysate is treated with a nuclease.

94. (Currently Amended) The method of claim 70, wherein said purified adenovirus composition for therapeutic use comprises a pharmaceutically acceptable buffer.

95. . . . (Currently Amended). The method of claim 94, wherein said purified adenovirus composition for therapeutic use provides a unit dose of between  $10^3$  and  $10^{15}$  PFU/dose.

Application No.: 10/033,571

Docket No.: 29853/37702

96. (Currently Amended) The method of claim 94, wherein the purified adenovirus composition for therapeutic use provides a unit dose of between  $10^{10}$  and  $10^{14}$  PFU/dose.

97. (Currently Amended) The method of claim 70, wherein said purifying is characterized by a process that includes at least one chromatography step to provide a pharmaceutically therapeutically acceptable composition.

98. (Currently Amended) The method of claim 70, wherein said purifying is characterized by a process that includes a single chromatography step to provide a pharmaceutically therapeutically acceptable composition.

99. (Withdrawn) The method for making a purified adenovirus composition for therapeutic use comprising:

- a) growing host cells in a media comprising glucose;
- b) infecting said host cells with an adenovirus;
- c) lysing said host cells by a lysis method other than freeze-thaw to produce a lysate comprising said adenovirus composition; and
- d) purifying adenovirus from said lysate to provide a purified adenovirus composition for therapeutic use.

Application No.: 10/033,571

Docket No.: 29853/37702

100. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use comprises 70% +/- 10% of the starting PFU.

101. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use comprises a substantially purified therapeutic adenovirus composition.

102. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml.

103. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.2 ng/ml.

104. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has an A<sub>260</sub>/A<sub>280</sub> ratio of between about 1.2 and 1.3.

105. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has an A<sub>260</sub>/A<sub>280</sub> ratio of 1.27 +/- 0.03.

Application No.: 10/033,571

Docket No.: 29853/37702

106. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a contaminating nucleic acid concentration of less than 0.8 ng/ml and an A<sub>260</sub>/A<sub>280</sub> ratio of between about 1.2 and 1.3.

107. (Withdrawn) The method of claim 99, wherein the purified adenovirus composition for therapeutic use has a BSA content below the detection level of a western blot assay.

108. (Withdrawn) The method of claim 99, wherein the media is serum-free.

109. (Withdrawn) The method of claim 99, wherein the host cells are grown in a bioreactor.

110. (Withdrawn) The method of claim 99, wherein the host cells are grown on microcarriers.

111. (Withdrawn) The method of claim 99, wherein said purified adenovirus composition for therapeutic use comprises an adenoviral vector encoding an exogenous gene construct.

Application No.: 10/033,571

Docket No.: 29853/37702

112. (Withdrawn) The method of claim 111, wherein said exogenous gene construct is operatively linked to a promoter.

113. (Withdrawn) The method of claim 112, wherein said promoter is SV40 IE, RSV LTR,  $\beta$ -actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

114. (Withdrawn) The method of claim 111, wherein said exogenous gene construct encodes a therapeutic gene.

115. (Withdrawn) The method of claim 114, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl*, antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-1, MEN II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, GM-CSF, G-CSF, thymidinc kinase or p53.

116. (Withdrawn) The method of claim 115, wherein said therapeutic gene encodes p53.

117. (Withdrawn) The method of claim 99, wherein said purified adenovirus composition for therapeutic use is a replication-incompetent adenovirus.

Application No.: 10/033,571

Docket No.: 29853/37702

118. (Withdrawn) The method of claim 117, wherein said purified adenovirus composition for therapeutic use is lacking at least a portion of the E1-region.

119. (Withdrawn) The method of claim 118, wherein the purified adenovirus composition for therapeutic use is lacking at least a portion of the E1A and/or E1B region.

120. (Withdrawn) The method of claim 99, wherein said host cells are capable of complementing replication.

121. (Withdrawn) The method of claim 99, wherein said host cells are 293 cells.

122. (Withdrawn) The method of claim 99, wherein said lysate is treated with a nuclease.

123. (Withdrawn) The method of claim 99, wherein said purified adenovirus composition for therapeutic use comprises a pharmaceutically acceptable buffer.

Application No.: 10/033,571

Docket No.: 29853/37702

124. (Withdrawn) The method of claim 123, wherein said purified adenovirus composition for therapeutic use provides a unit dose of between  $10^3$  and  $10^{15}$  PFU/dose.

125. (Withdrawn) The method of claim 123, wherein the purified adenovirus composition for therapeutic use provides a unit dose of between  $10^{10}$  and  $10^{14}$  PFU/dose.

126. (Withdrawn) The method of claim 99, wherein said purifying is characterized by a process that includes at least one chromatography step capable of providing a purified therapeutic adenovirus composition.

127. (Withdrawn) The method of claim 99, wherein said purifying is characterized by a process that includes a single chromatography step capable of providing a purified therapeutic adenovirus composition.

128. (Withdrawn) The method of claim 99, wherein the lysis method other than freeze-thaw is a process that includes hypotonic solution, hypertonic solution, impinging jet, microfluidization, solid shear, detergent, liquid shear, high pressure extrusion, autolysis or sonication to produce a crude lysate composition comprising adenovirus.